STUDIES ON THE POSTEMBRYONIC DEVELOPMENT OF HYALELLA AZTECA (SAUSSURE)[1]

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Hyalella azteca is a common and widely distributed fresh-water amphipod crustacean. The genus Hyalella is the only one of the family Talitridae occurring in the fresh waters of America. The species *H. azteca* described by Saussure in 1858 from Vera Cruz, Mexico, where it was originally found among the ruins of the Aztec Indians, is widespread in North and South America, and has been cited in the literature more often under the synonyms *Hyalella knickerbockeri* (Bate) or *H. dentata* Smith.

Holmes (1902, 1903) made observations on *H. dentata* Smith to determine the mode of sex recognition, food habits, thigmotaxis, phototaxis, and reaction to pressure. Weckel (1907) discussed the synonymy of this species and gave a minute description of its characteristic external features. Embody (1911), interested in propagating amphipods as food for fishes, made a study of the distribution, food, and reproductive capacity of four common fresh-water amphipods, including *Hyalella knickerbockeri*. He states that this amphipod breeds for 152 days during the warmer months of the year and averages about 18 eggs (15 times in 152 days) per brood.

Jackson (1912) investigated the distribution and habits of this species, also its color, size, moulting, effects of starvation on moulting, breeding, locomotion, and enemies. In his paper there is no mention of temperature, and the age of the animals was judged by their size. In 1915 Phipps made an experimental study of the behavior of amphipods with respect to light intensity, direction of rays and metabolism. *Hyalella knickerbockeri* was one of the animals included in his study. Gaylor (1922) reported on the life history and productivity of this species, and recently, the effects of population density upon its growth, reproduction and survival were studied by Wilder (1940).

In the above-mentioned investigations of *Hyalella azteca* no account exists of the morphological changes that occur in its postembryonic development, a necessary preliminary to experimental studies for which this animal seems suitable. In fact, few amphipods have been so studied. The work of Sexton (1924) on *Gammarus*

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chevreuxi is undoubtedly the most outstanding contribution to our knowledge of the postembryonic development of amphipods. Sexton raised the amphipods individually from birth and studied their complete series of moults. When her work was begun in 1909 nothing was known as to the length of life of an amphipod, its moulting periods, incubatory period of the eggs, time required to reach maturity, etc. In addition to answering these questions for several species, she found the number of growth stages from hatching to sexual maturity to be different not only in the different genera, but different even in the various species of any one genus. *Gammarus locusta* lays eggs after the twelfth moult, *G. chevreuxi* after the seventh, and *G. pulex* after the tenth moult.

With the hope of adding to the knowledge of amphipod development, and keeping in mind broader biological problems, the question of growth and differentiation of *Hyalella azteca* was investigated according to the following plan:

1. Growth, from hatching to sexual maturity, considered with respect to the number of moults and the morphological differences between the instars.
2. Correlation between the differentiation of external secondary sex characters and the development of the gonads.

Materials and Methods

The Hyalellas were obtained from Mr. Eugene W. Surber, in charge of the Fish Hatchery of the U. S. Fish and Wildlife Service at Kearneysville, West Virginia. They are descendants of a stock originally collected by Mr. Surber in a Mississippi River slough near St. Charles, Missouri.

The study of growth, from hatching to sexual maturity, was investigated according to the following procedure: Young Hyalellas were taken on the day they emerged from the brood pouch of the mother and placed in individual culture dishes. For the newly-hatched animals ordinary Syracuse dishes were used. Bits of freshwater plants, such as Elodea and Vallisneria were added to the tap water and sand in the culture dishes. When the animals grew larger and there was danger of their crawling over the edge, larger dishes were substituted. The young animals were kept in a dark room where a constant temperature of 16 to 18° C. was maintained. Cultures were examined daily, and the moults preserved separately in vials of 5 per cent formalin until complete series from birth to maturity had been collected. Niagara Sky Blue, an aniline dye excellent for chitin, was used in staining the moults, and permanent mounts were made in Diaphane.

As soon as the first formation of eggs was observed in the young developing female, a male from a stock culture of mating males was added to her culture dish. Both the date of pairing and date of egg laying were recorded. The number of moults that preceded each of these events was likewise noted. Further observation was made in regard to the development of these eggs and the number of young produced in first broods.

When males could be identified by their large gnathopods, females (from the stock culture) with ripened eggs in their ovaries were added. Females were used that had just released young from their brood pouch and had new eggs ready to be fertilized. After pairing had taken place and eggs passed into the brood pouch, the eggs were observed for development, as this was the only means of determining whether or not the males were mature.

A comparison of the moults was made to study the earliest appearance and subsequent differentiation of secondary sexual characters. Brood plate development was considered in relation to growth of the ovary. Changes in the size and shape of plates as well as the time of the appearance of hairs were recorded. In the male, gnathopod differentiation was studied in its relation to the mating period. In addition, moults were studied for antennal growth and development of dorsal teeth.

General Observations

A. Description of Hyalella azteca (Saussure)[2]

The body of *Hyalella azteca* is elongated and laterally compressed, with the first thoracic segment fused to the head. The length of the animal is 4 to 6 mm. The thorax is composed of seven and the abdomen of six segments; the telson is small and entire. The eyes are sessile, compound, and round or nearly so. Antenna I is shorter than antenna II and is without an accessory flagellum; these features being characteristic of the family Talitridae. The peduncle of antenna I consists of three joints; the first and second joints are about equal in length and slightly longer than the third; the flagellum is composed of seven to nine joints, and is about twice as long as the peduncle. The peduncle of antenna II consists of five joints, on the second of which is located the antennal gland. The two distal joints of the peduncle are elongated and nearly equal. The number of joints in the flagellum of antenna II varies from eight to fifteen. Maxillae and maxillipeds will not be described as the study of their development does not enter into this investigation.

All thoracic legs, except the first and last pairs, bear gills on the inner side of the first joint. It is of interest to note that *Hyalella azteca* has two kinds of gills, sternal and coxal. Small lateral sternal gills are located on the thoracic segments III to VII inclusive, and the coxal gills project from the inner surface of the first joint of the thoracic legs II to VI inclusive. The first two pairs of thoracic legs differ from the others and are called gnathopods; the remaining five pairs are more or less similar in structure and are termed peraeopods.

Extending from the first three abdominal segments are three pairs of pleopods, each pleopod consisting of a long basal joint and two multiarticulated, setose rami. Pleopods are employed not only in swimming, but to direct water toward the gills. As the pleopods are constantly in motion even when the animal is resting, the gills and the developing embryos in the brood pouch are always aerated. A dorsal tooth projects from the posterior edge of each of the first two abdominal segments. The three posterior abdominal segments each bear a pair of uropods. These are directed backward and fitted for springing. The first and second pairs of uropods are biramous, and the third pair is uniramous.

1. *Males*

Males are distinguished from females by their larger size and by their second gnathopods. The propodus of the male's second gnathopod is very large, and the

[2] Smith gives a good picture of *Hyalella azteca* (=*Hyalella dentata*) in *Rep. U. S. Fish Com.*, 1872–73 (1874), p. 645, pl. 2, fig. 8.

palm is subchelate, in contrast to the female's which retains its juvenile small and chelate form. The testes, located beneath the heart, are much elongated and taper at each end. The male ducts open by papillae on the ventral side of the last thoracic segment.

2. *Females*

In females ova can be distinctly seen and approximately counted as they lie in the ovaries which are ventral to the heart. The paired tube-like ovaries extend from the second thoracic segment to about the middle of the seventh segment. When filled with eggs, they appear to terminate abruptly at each end. The narrow oviducts open at the base of the fifth coxal plates so that when the eggs are released they are caught in a brood pouch formed by the lamellae. Only during the passage of eggs can the oviducts be seen. In passing down, the eggs lose their ovoid shape and resemble a worm pressing its way forward. Immediately upon reaching the brood pouch the eggs assume their former shape.

The mature female can also be distinguished by lamellae, which occur on the inner surface of the coxal joints of thoracic legs II to V inclusive. The edges of the lamellae, except the posterior edges of the fourth pair, are bordered with hairs that are hook-shaped at their distal end. Hairs from adjacent lamellae entangle about each other and help to hold the eggs in the pouch.

B. Observations on mating

One of the characteristic features of the Amphipoda is their habit of pairing. Smallwood (1905) observed that even the sand fleas, *Orchestia palustris,* retain the habit of pairing, a very awkward process on land. Gaylor (1922) observed that Hyalellas pair for as many as seven days before copulation. In his discussion of sex recognition, Holmes (1903) states that pairing of the animals is determined by the reaction of the animals when they collide with each other.

Pairing is followed by the shedding of the female's moult, a necessary preliminary to copulation. Normally, the oviducts are closed externally, and temporary openings are only present after the moult when the cuticle is soft. The male then deposits sperm in the brood pouch around the orifices of the oviducts, and the eggs are fertilized as they enter the pouch. It may be mentioned here that the female Hyalella is able to release eggs into the brood pouch without the influence of the male, contrary to the statement of Embody. However, as such eggs are unfertilized, they apparently disintegrate and are eventually lost.

After copulation the animals cease swimming together. When a female bearing eggs in her brood pouch is found paired it is evident that the embryos in the pouch are well developed and will hatch in a few days, and the male is to become father of the next brood. Usually the ovaries are by this time filled with ripening eggs.

At the temperature at which Hyalellas were kept, about 21 days were required for development of the eggs in the brood pouch, i.e., from fertilization to hatching. After hatching, the young remain in the brood pouch for one to 3 days. One can observe the movement of their pleopods within the mother's pouch.

When ready to emerge, the young crawl to the front of the pouch which is open. After reaching the edge, they are abruptly thrown off as the mother makes a sharp turn in her swimming movements. If the mother is again paired, the young do not remain in the brood pouch very long; the jerky movements of the male cause them to make an early exit. Some young were seen making their exit through the floor of the brood pouch by pushing the lamellae aside and tearing the hairs.

Instars Described in Terms of Moults

A. Four stages of development

Newly-hatched Hyalellas possess all the appendages of the adult, except the secondary sexual characters. Naturally, many modifications occur during the growth process. Unlike the amphipod, *Pontoporeia affinis,* in which the young possess male characters (Segerstrale, 1937), Hyalella young may be said to resemble the adult female, for the reason that the young have slender gnathopods like the female. Later, the males are distinguished by the increasing size of the propodus of the second pair of gnathopods.

In describing the growth stages of Hyalella, the instars may be conveniently divided into four characteristic periods: the Juvenile, the Adolescent, the Nuptial, and the Adult.

Table I

First occurrence of pairing determined for 20 males and 14 females in relation to instar age

Instar	5	6	7	8	9	10	11
Males.......	0	1	2	12	5	0	0
Females.....	0	1	0	13	0	0	0

The *Juvenile Period* includes the first five instars. First instar is that period extending from the time the animal emerges from the brood pouch to the time it sheds its first moult, assuming as seems likely, that no moult takes place while the young are still in the pouch. During the first five instars the sexes cannot be distinguished. The head of the newly-hatched animal is large in proportion to the rest of its body. Growth is slow and gradual, and the first antenna adds a maximum of three joints. In size and shape the propodi of the first and second gnathopods are similar. Dorsal teeth make their appearance in the third moult. With the shedding of the fifth moult the animal ends its Juvenile Period.

The *Adolescent Period,* including instars 6 and 7, shows an increased rate of growth in the entire animal. The sexes can now be distinguished. The propodus of the male's second gnathopod is much enlarged and the palm is transverse. The animals that retain the slender propodus at this period turn out to be females (with the exception of some retarded individuals). The paired tube-like gonads become more prominent and surrounded by adipose tissue. In some of the males the digestive tube, which is beneath the gonads, curves noticeably downward in the thoracic region and is lowest in the fourth, fifth, and sixth thoracic segments. In the seventh instar some of the females show the first formation of a few small eggs in the region of the fourth and fifth thoracic segments. Small brood plates are present but hairs are lacking on their borders. Another joint is added to the first antenna, and the fourth joint has again elongated and is ready to constrict.

The *Nuptial Period* designates the eighth instar during which the animals usually pair for the first time (Table I). Males pair almost as soon as the moult is shed, while the females generally pair toward the end of the period when the eggs in their ovaries have fully matured.

The animals are very much larger and the propodus of the male's second gnathopod is greatly enlarged with a distinct subchelate palm. One or two joints are added to the first antenna. The female's lamellae are turned back at the edges and a few hairs are present in the eighth moult which normally marks the end of the Nuptial Period.

The *Adult Period* occurs after the eighth ecdysis. Eggs are released into the brood pouch formed of fully developed lamellae bordered with hooked hairs. The eggs are fertilized by the sperm previously deposited around the orifices of the oviducts. Moult IX has an additional first antennal joint. When the ninth moult has been shed the animals have not attained full size. Growth continues beyond the tenth instar, but detailed observations were not carried on beyond this point.

TABLE II

Sample record showing duration of instars in days, Brood C, 12 individuals, emerged 12/8/42

Animal Instar......	1	2	3	4	5	6	7	8	9	10
♀C1..............	8	6	13	12	11	10	16	(died)		
♂C2..............	8	17	5	10	15	11*	17	19		
♀C3..............	8	5	9	9	10	9	7	10*		
♀C4..............	9	26	12	10	8	12*	22			
♂C5..............	9	14	7	10	7	9	20	16*		
♂C6..............	9	13	11	8	9	9	10	12*	30	
♀C7..............	9	14	9	9	9	9	9	12*	25	
♂C8..............	10	14	9	8	9	10	12*			
♀C9..............	10	16	15	4	8	9	11	16*		
♂C10.............	11	15	10	6	9	10	9	24*	23	
♂C11.............	11	15	10	8	10	8	10	10	18*	
♂C12.............	12	12	8	10	9	9	12	17*		

The asterisk indicates the instar during which the animals paired for the first time.

Table II shows the duration of instars in days of the animals in Brood C.

B. General growth rate

At first growth is slow, moults I and II appearing to be the same size. The duration of the second instar is quite long; this is evidence of slow growth. In Figure 1 the time in days between moults is graphically represented for seven broods. Instars 3, 4, and 5 are short and during this period growth is rapid. Following moult V, differentiation of secondary sexual characters sets in. As the animals age they shed their moults less frequently. It is a known fact that young animals moult more frequently than do adults. Adult females moult more often than do adult males.

Body size in terms of moult length was determined for three representative males and two females, the measurements of the moults being made from behind the head to the tip of the telson. The averages are seen in Figure 2. This graph shows that moults II, VI, and VII are larger in the females studied than in the males. However, general observations indicate that after attaining maturity the male grows at a faster rate than the female and as adult is characteristically larger

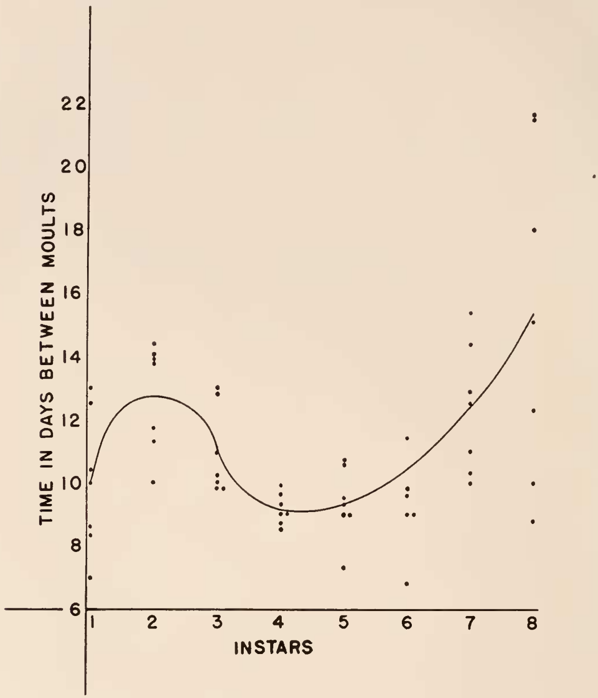

FIGURE 1. Time in days between moults measured for seven broods.

than the adult female. This does not appear on the graph since detailed studies in this case did not go beyond the eighth instar.

C. Growth of the antennae

Newly-hatched Hyalellas have six joints in the first antenna. The three proximal joints constitute the peduncle, and the distal three the flagellum. During the process of growth the number of peduncular joints remains constant while the flagellum develops four to six new joints. The actively growing region ("growth center") is the first proximal joint of the flagellum. Growth can readily be studied through the series of moults from any one animal. A number of series were studied to compile the following table which refers to number of joints in the entire first antenna:

Moult I.	6 joints; No. 4 is long.
Moult II.	6 joints; No. 4 is long. Or 7 joints with No. 4 short and lacking setae. The constriction has just occurred, and setae which are normally found on the distal edge of each joint, have not yet developed.
Moult III.	7 joints; No. 4 is short and lacking setae.
Moult IV.	7 joints; No. 4 is long, and has setae. Or 8 joints with No. 4 short and lacking setae.
Moult V.	8 joints; No. 4 is long, and has setae. Or 9 joints with No. 4 short and lacking setae.
Moult VI.	9 joints; No. 4 is long, and has setae. Or 10 joints with No. 4 short and lacking setae.
Moult VII.	10 joints; No. 4 is long, and has setae.
Moult VIII.	11 joints; No. 4 is long, and has setae. Or 12 joints with No. 4 short and having setae.
Moult IX.	11 joints; No. 4 is long, and has setae. Or 12 joints with No. 4 long and having setae.

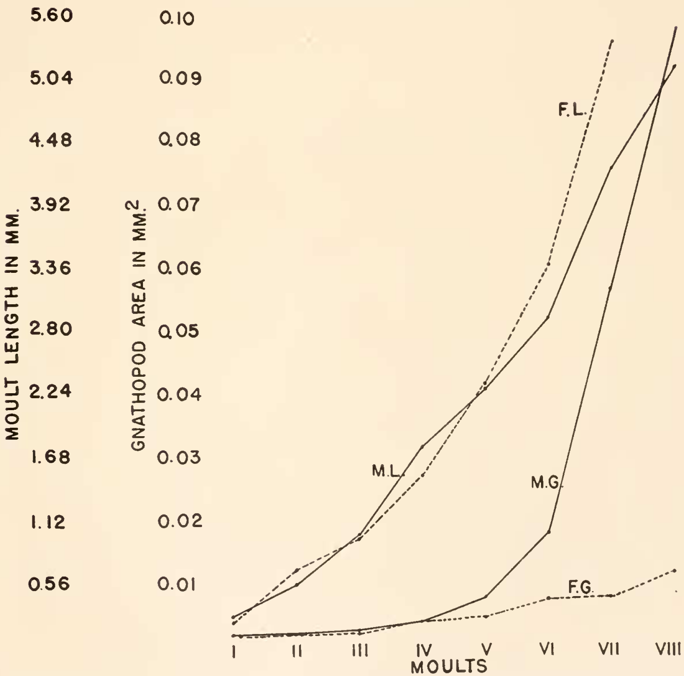

FIGURE 2. Growth in length of body of *Hyalella azteca* plotted against growth in area of gnathopods (propodus and dactylus). M.L.—length of male moult. F.L.—length of female moult. M.G.—area of male gnathopod. F.G.—area of female gnathopod.

In the early stages of development growth is relatively slow. With the shedding of two moults only one joint is added. From Moult V onward, joints are added at the rate of one per moult. Plate I is a representative series of drawings from moults shed by the animal D8. The outside view of the right first antenna is drawn to scale.

It cannot be said that any particular moult always has a certain number of joints, because the growth rate varies in different animals. Sometimes in the same moult one of the first antennae may have one joint more than the other first antenna (Pl. III). In such a moult it will be noted that in the one case the first proximal flagellar joint has lengthened and constricted, and in the other case, the corresponding joint has lengthened; due to a slower rate of growth, it has not yet constricted. In general, however, the number of flagellar joints is helpful in ascertaining the age and instar of the animal. An animal with a six-jointed first antenna cannot be beyond its third instar; if the animal has nine or ten joints, it is not yet mature, but is passing through one of the adolescent instars.

Second antennae—method of multiplication

As in the case of the first antenna, the "growth center" or region of most active growth of the second antenna is located in the first proximal joint of the flagellum. There are five joints in the peduncle and therefore the first joint of the flagellum is No. 6.

Moult I has a total of nine joints and this number does not increase in the second and third moults, although joint No. 6 elongates preliminary to constriction. Moult IV has an additional joint. In moults V to IX the number of joints steadily increases from 11 to 15.

In *Hyalella azteca* only one region of growth could be detected in the antennae, but in some amphipods more than one growing region is present. Segerstrale (1937) found this condition in the male *Pontoporeia affinis*. In the beginning, only the proximal joint of the flagellum divided, but in the third last stage, four of the more distal joints divided, and in this same stage the proximal joint divided three times. In the stage before the last, 15 proximal flagellar joints divided, so that the last stage of the male has a flagellum of 41 joints. The female of this species is distinguished by a shorter flagellum. Only the proximal joint divides. In the stage before the last there are ten joints, and the last stage shows no increase.

In *Gammarus chevreuxi* Sexton (1924) found that the proximal joint of the first antenna divides, usually into two portions, and the distal one which is the new joint she terms the "undivided primary joint." At the next moult the undivided primary joint elongates and divides into two portions, the distal part or true primary joint and the proximal portion which may be called the secondary joint. The process is repeated in each stage, the last formed primary joint subdividing and giving rise to a secondary joint; the formative zone dividing and giving off one or

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Plate I

Development of first antennae is shown above. The right first antenna is drawn to scale in outside view from the first eight moults shed by one animal. Arrows indicate growth pattern, i.e. the manner in which successive new joints are added by division of the fourth joint. Development of propodus and dactylus of first gnathopods of a female is shown below. Drawings made to scale from the first eight moults shed by a female.

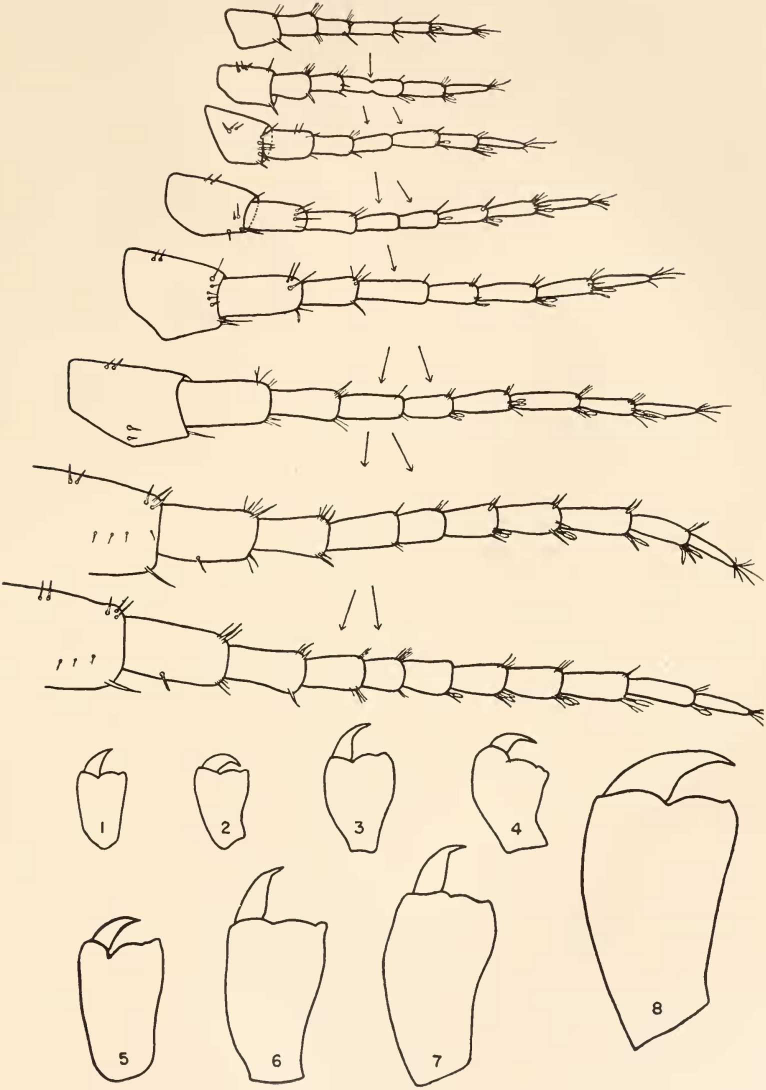


PLATE I

more undivided primary joints. Sexton also mentioned that sometimes more than one primary joint may be formed in the older animals. In *G. chevreuxi* the second antenna has a shorter flagellum than that of antenna I, and is slower also in the rate of growth.

Growth of the first antenna of *G. chevreuxi* is more rapid than that of *H. azteca*, for in moult I of the former there are seven joints in the antenna and in moult VIII of the same animal there are 23 joints. In moult I of *H. azteca* there are six joints in the first antenna and in moult VIII there is a maximum of 12 joints.

While the second antenna of *G. chevreuxi* is slower in its rate of growth than the first antenna, the rate of growth in the second antenna of *H. azteca* is about equal to that of its first antenna for six additional joints are usually present at the eighth moult.

Development of Secondary Sexual Characters

A. Gnathopods

In the early instars the propodus of the first and second gnathopods in both sexes is approximately the same size, but distinctly different in form. The palm of the propodus of first gnathopods is transverse and concave at the middle. Although this propodus increases in size with the age of the animal, it does not alter in form in either sex. A series of propodi of first gnathopods of a female, C13, is shown in Plate I. These were drawn to scale from the mounted moults of the animal. The first gnathopods of the male have similar propodi.

In contrast, the second gnathopods become sharply differentiated as the sexes mature. In both males and females the palm of the propodus of the second gnathopods is chelate during the juvenile period, and in the female the juvenile form is retained throughout life, even after the propodus has become long and slender. The complete series of propodi of second gnathopods of C13, also drawn to scale, are shown in Plate II and indicated by the letter "F."

After the fifth moult, the propodus of the male's second gnathopod gradually changes both in size and form. The palm loses its chelate form and gradually becomes transverse. From about the seventh moult on, the palm assumes a pronounced subchelate form. The dactylus of the male curves more strongly to fit the changing form of the palm. In Plate II the series of propodi indicated by the letter "M" are from male second gnathopods. All are from the male C12, except No. 3, No. 5, and No. 6 which are from the male B1. Moults III and V of C12 were badly torn, and moult VI was unusually small and not representative of a typical sixth male moult, so in the drawing the propodus from B1 was substituted.

Hundreds of male moults had been collected in an attempt to secure a complete series from one animal. Although Hyalellas do not eat their moults, they sometimes tear them badly in the process of moulting. In many cases, moults that had been preserved were found to lack both propodi from the second gnathopods, the very feature that was most essential to this study. In moulting, the male has to pull his immense propodus through the narrow carpus and in the struggle the

Plate II

Development of propodus and dactylus of the second gnathopods. Drawings made to scale from the first eight moults of a female (F) and male (M).

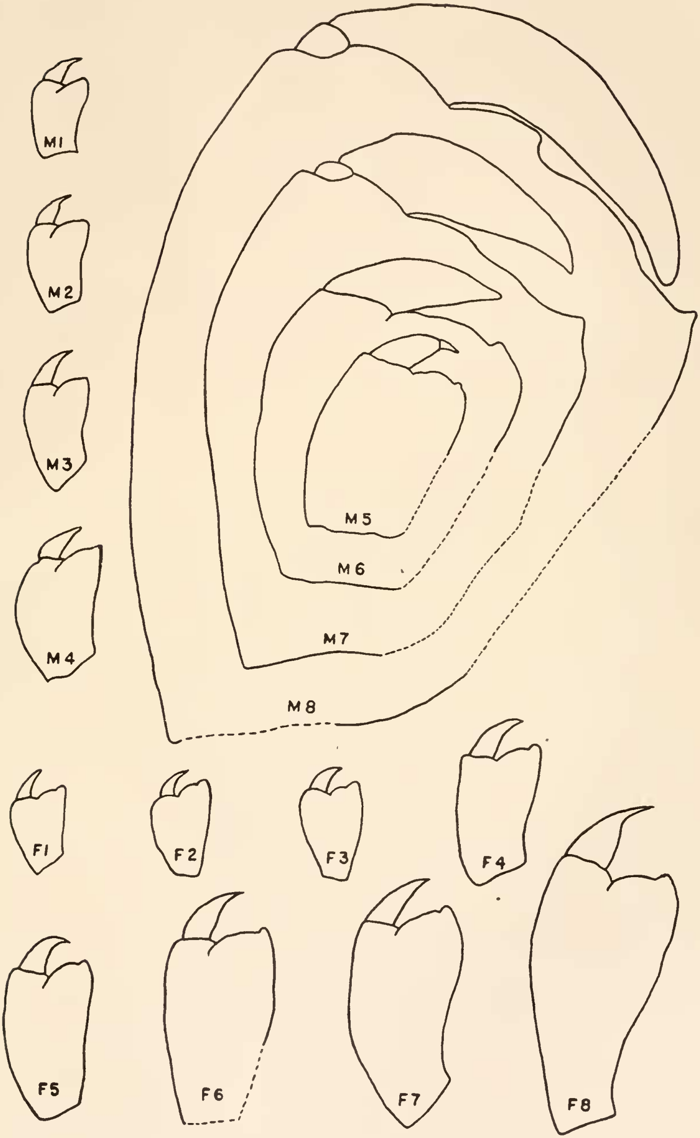


PLATE II

propodi are often torn off. In nearly every propodus that is retained the sternal border is torn.

To obtain some idea of the increase in area of the propodus of both males and females, series of drawings to scale were made, and the actual area of the gnathopod was calculated from these drawings. A complete series of propodi from one female was used, but as there was no complete series of male propodi, an average obtained from the propodi of three males was used in determining area. The data are presented in Figure 2.

The propodus of the male in moult V is 4.7 times the area of the same propodus in moult I; in moult VI, it is 11.8 times, in moult VII, 37 times, and in moult VIII, 64 times. The female propodus shows a gradual increase; in moult VIII it is 8 times the area of the same propodus in moult I (Pl. II).

In *Hyalella azteca* the gnathopods differentiate as external secondary characters before the animals are sexually mature. When males could be identified by their gnathopods as early as the sixth instar, mature females with ripe eggs in their ovaries were added. At this stage the males were not mature, because they did not pair. When those same females were removed and placed with mature males from the stock culture, pairing immediately took place and was followed by copulation.

After the young males shed their next moult and were then in their seventh instar, mature females were again added. In a very small percentage of cases pairing took place. In general, pairing took place after males had shed seven moults. The fact that young hatched from those eggs shows that they were fertilized. Although males may be regarded as sexually mature in their eighth instar, in five cases males first paired during the ninth instar (Table I). In these instances the females that were added during instar 8 may not have been in the right physiological condition for mating.

If we consider pairing as an indication of maturity, both sexes are normally mature in the eighth instar. On the other hand, if the instar during which ova deposition occurs is taken as the first sign of sexual maturity then the female is mature in the ninth instar, because her eighth moult must be shed before eggs are released. The male, in the latter case, may be said to mature one instar before the female, because he is capable of fertilizing eggs immediately after pairing and before his eighth moult is shed.

The first gnathopods of the male Hyalella are employed in carrying the female and are structurally adapted for this purpose. The fact that the second gnathopods enlarge when the male approaches maturity seems to indicate that they are necessary for sex recognition. Males need them to actively resist each other, and females in lacking them are not able to resist being carried, as described by Holmes (1903). Sexton observed that *G. chevreuxi* male carries the female with his second gnathopods, and Heinze (1932) stated that *G. pulex* male uses his first gnathopods for this purpose.

After reaching maturity, the male continues to grow, and the second gnathopods increase in size proportionately, until the maximum adult form is reached.

B. Lamellae

Lamellae, characteristic of adult females, are entirely lacking in the young animals. The sixth moult of females has small, incompletely developed lamellae; the seventh has them slightly enlarged. In moult VIII the edges of the lamellae are turned backward and bordered with a few scattered hairs. Moult IX contains the fully developed lamellae bordered with hairs; this is the first adult moult of the female.

The hairs differ from the long, straight hairs found in Gammarus and certain other amphipods. In Hyalella, as in other members of the family Talitridae, the basal portion of the hair is slightly enlarged, and the distal end is swollen and curved around in the shape of a hook. The hooked ends join with hairs from opposite or adjacent lamellae to help hold the developing eggs in the pouch (Pl. III). When eggs are first deposited in the pouch, opposite lamellae overlap each other. In later stages, when the embryos are large, the lamellae are fully stretched out and only the hairs cling together. Perhaps the increased weight or the movement of the young push the lamellae out to their full extent. Small, young females generally have fewer eggs than older females which are larger, and the size of the lamellae is in proportion to the size of the animal. Four to 13 young were produced in first broods.

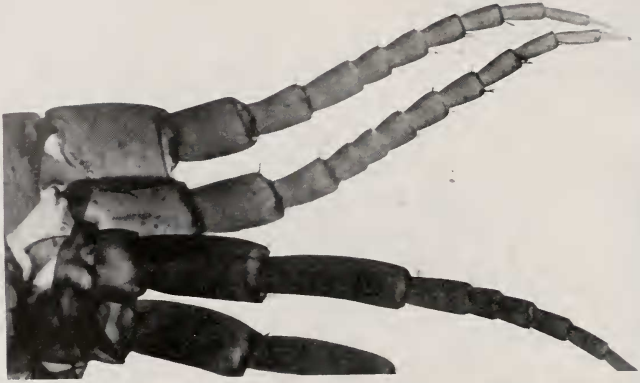
The fourth pair of lamellae is smaller than the three anterior pairs and the posterior margin, which is slightly chitinized, is curved upward. Hairs are lacking along that same border. Due to the upward curvature of the lamellae, it may be that hairs are not necessary. A few spines are located on the external side of the curved portion.

Development of hairs is in some way correlated with maturing of the eggs, for they appear and function for the first time when the first eggs are released.

The development of lamellae in Hyalella is similar to that of other amphipods. Sexton reported that in *G. pulex* where the cuticle is firmer and more substantial than in *G. locusta* or *G. chevreuxi,* she has been able to trace brood plates in four growth stages preceding maturity. First they appear as minute, rounded leaf-like plates with margins entire; in the next moult they have lengthened and increased to three times the size; rudimentary hairs make their appearance on the next moult, and in moult X (the moult after which eggs are laid) brood plates are very large, chitin is hard, and rudimentary hairs more numerous. In the next stage when sexual maturity is reached, the brood plates are fully developed with long fringing hairs. In *G. locusta* and *G. chevreuxi* Sexton traced brood plate development in three stages before sexual maturity.

Heinze, too, from his study of *Carinogammarus Roeselii* Gerv. concluded that the first anlagen of oostegites appears several moults before the female becomes mature. In fact, he found the first pair of lamellae on the third segment when the animal was 4.5–5 mm. Heinze does not mention the instar of the animals, but gives their length in millimeters. When the female is 5–5.5 mm. long the four pairs of brood plates are present, the fourth pair on segment 6 being the narrowest. The closer the female approaches maturity, the larger the brood plates become. In stages 7.5 mm., 8 mm., 8.5 mm. the hairs are still lacking, and in the next stage, bordering of the brood plates with hairs precedes copulation. In *G. pulex* Heinze found similar conditions, the first pair of brood plates appearing when the animal is 4.5 mm. But corresponding to the larger size of the female, the oostegites in their development were more advanced.

Segerstrale reported that the first anlagen of brood plates in *Pontoporeia affinis*



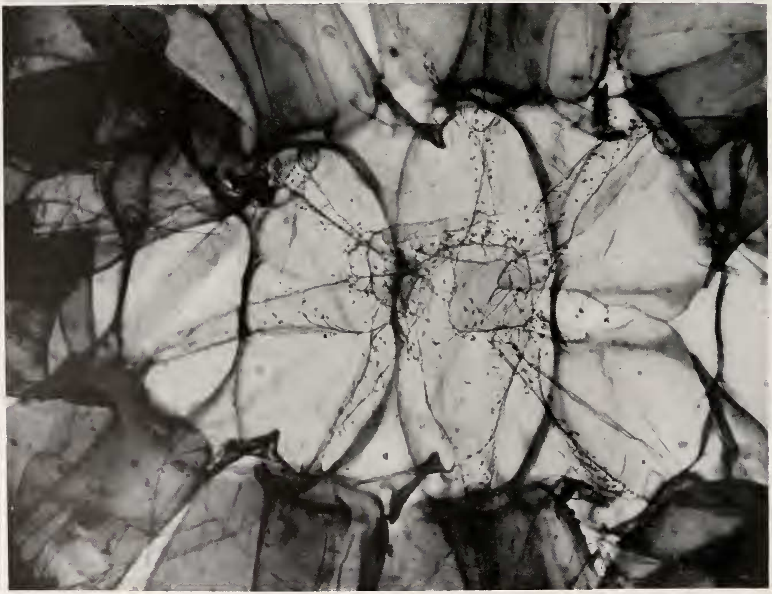


PLATE III

FIGURE 1. A case of unequal growth of the first antennae. A constriction has appeared in the first proximal flagellar joint of the right antenna, while the same joint in the left antenna has elongated but not yet constricted. × 68.

FIGURE 2. Photomicrograph of female moult to show brood plates with interlocking hairs as seen from within. × 68.

occurred in females that were 5.3–5.5 mm. in body length. They appear as small rounded structures which expand by degrees so that in the stage before the last they show approximately the final form. The enlargement of the area occurs in the greatest degree only during the last moult.

Retarded Individuals

During the course of this investigation, it soon became apparent that all the animals did not mature at the same time. This was first noticed in the animals that were raised individually. In the eighth instar the animals generally paired, showing that they were sexually mature. About one or two in some broods failed to pair at this time, due to their immature condition. For the present, since their precise nature is unknown, these slow-developing animals will be termed "retarded individuals." At a stage when their siblings could readily be distinguished by their gnathopods, and also by eggs in the case of the females, these retarded individuals, while possessing the slender gnathopods of the female, lacked visible eggs. Retarded individuals are smaller than the rest of the animals in the same brood and grow at a slow, steady rate. At a much later stage some of them gradually developed male gnathopods and eventually paired. Two others after the tenth moult and therefore during the eleventh instar, developed eggs and later paired. One of them lost its first brood of eggs, but its second brood, which had been fertilized by a retarded, but now mature male, developed into young in the normal length of time. However, none of the young reached maturity.

Two retarded individuals, aged 67 days (the rest of their brood had matured at 40 days), when sectioned and stained, showed a few very small eggs in their gonads. Another, having female gnathopods but lacking eggs, was dissected. It was found to have large, normal brood plates that lacked hairs.

It is possible that the retarded individuals are similar to the intersexes described so completely by Sexton. A thorough study of them, outside the scope of the present investigation, would have to be made to determine their exact status. It would be interesting to learn whether a deficiency, hormonal or nutritional, prevents maturation of the gonads at the normal time, whether their genetic constitution warrants a later maturation, or whether retardation may be caused by a state of depression induced by unfavorable external factors. A study of this special problem is now under way.

Summary

Hyalella azteca grows well under laboratory conditions. Like other amphipods, the frequency of its moulting depends upon several factors, chief of which is temperature. At low temperatures ecdysis is delayed. Frequency of moulting in developing animals is no indication of sex, as it occurs at varying time intervals in both sexes. The young moult more often than do the adults. In reaching the time for first pairing, the number of moults is more important than the age, in days, of the animal. First pairing generally occurs in the eighth instar.

In general, growth at first is slow. After the secondary sex characters first become apparent (Instar 6) growth becomes more rapid.

Centers of more active growth are present in certain regions, such as the second gnathopod of the male, and the proximal joint of the flagellum in both pairs of

antennae. In the female the fifth thoracic segment appears to be a "growth center," for eggs begin forming in this region. No doubt, there are regions of active growth throughout the body, but the above are the most prominent.

Animals during any instar are not the same size. Males particularly vary in size, although they may mature after the same moult. Retarded individuals, animals that pair much later than the normals, are fairly common. It is possible that some of them never become sexually mature.

The external secondary sex characters, lamellae and gnathopods, make their appearance several moults before the animals pair. Lamellae grow gradually, and reach their full size and become bordered with hooked hairs when the first eggs are released.

First gnathopods in both sexes are similar in size and form. Second gnathopods of the female retain their juvenile form, although they increase in length. Second gnathopods of the male develop a very large propodus with a strong dactylus and the palm of the propodus changes from chelate to transverse and then finally to subchelate.

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